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VEGETABLES AS A POSSIBLE FACTOR IN THE DISSEMINATION OF TYPHOID FEVER.

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Obviously a factor of great importance in the consideration of the prevalence and spread of typhoid fever is the viability of the *Bacillus typhosus*. Through what agencies the majority of cases contract infection is a mooted question by no means settled. Without going into such a discussion to any great length, it would seem that the dissemination of infection is due not so much to any article of food or drink as to the incidence of contamination. A study has been made of contaminated vegetables as one of the factors in transmitting typhoid infection, with additional reference to the longevity of the *Bacillus typhosus* in polluted soil.

Regarding the viability of the *Bacillus typhosus*, outside the human body, a large amount of experimental work has been done. The diversity of results has been in direct proportion to the number of investigators. Most of the work has been confined to soil, feces, and water.

Wurtz and Bourges in 1901 demonstrated that plants could be contaminated by infected soil. They recovered the *Bacillus typhosus* from vegetables grown under such conditions up to three weeks after the soil had been infected.

It is probable that much of the work done by early investigators in the study of the viability of the *Bacillus typhosus* was without value, because of the confusion of Eberth's bacillus with other organisms, which had in common with the *Bacillus typhosus* certain cultural or biological peculiarities. Admitting, however, that much of the earlier work is not to be unreservedly accepted, studies have been done in comparatively recent years by investigators, whose methods and results are unquestioned. Notably among these are Firth and Horrocks who worked upon the longevity of the *Bacillus typhosus* in soil. Their identification of the organism was complete. On ordinary soil, inoculated with an emulsion of the *Bacillus typhosus* in plain water, the ground being given no subsequent treatment except rain water in proportion to the natural precipitation, the organism was recovered throughout 67 days. In the same soil, nurtured with diluted sterile sewage, the longevity was 74 days. In the soil around an old drain, the organism was recovered 65 days

after inoculation. On soil kept perfectly dry and exposed to direct sunlight, the longevity was twenty days, with a total exposure to sunlight of 120 hours.

More recently Galvagne and Calderini, using most thorough methods of identification, determined the longevity of the *Bacillus typhosus* in a privy vault, in a barrel, and in soil. The longevity in the privy vault was 30 days, in the barrel 25 days. In feces spread upon soil, after 10 days in the vault, the duration of viability was 20 days on the surface and 40 days in the deeper layer, a total of 30 and 50 days, respectively.

Levy and Kayser report a case where the longevity of the *Bacillus typhosus* from feces in a naturally infected vault extended to over 5 months. This included a period of 14 days, during which time the infected feces had lain as manure in a garden.

Mair regained the organism after 84 days from soil inclosed by glass and with moisture added. From an aquarium containing fish, protozoal, and plant life with a bacterial count of 60,000 per cubic centimeter and exposed to sunlight during part of the day, Hoffman regained the *Bacillus typhosus* from water 36 days, and from mud at the bottom of the tank, 2 months after infection.

Such results as these seem ample refutation of the contention of those who claim that the *Bacillus typhosus* is an obligative parasite.

From lack of more definite methods our knowledge of typhoid infection in human beings is limited chiefly to various epidemiologic surveys. In arriving at conclusions as to the infecting agencies, the epidemiologist bases his findings on presumptive evidence, and, while his deductions may be clearly logical and convincing, still such a process of forming conclusions not infrequently admits of doubt as to the actual cause of infection. In reviewing the epidemiological data of different typhoid epidemics, the fact that the majority of typhoid cases, forming so-called "explosive" outbreaks, acquire infection through the ultimate sources of milk and water, becomes apparent. In the prosodemic cases, the part played by carrier infection, through contact with food, or other similar channels, has been more thoroughly appreciated in the last decade. Little attention has apparently been paid to the rôle of foodstuffs which are eaten uncooked, such as fruits and vegetables, in conveying infection acquired at the time of cultivation. In order to determine the possibility of this means of infection and to test the viability of the typhoid bacillus on garden truck, the following experiments were conducted in raising radishes and lettuce, fertilized with infected material.

METHOD OF INOCULATION.

Preliminary work was done in December, 1910, and January, 1911, under hothouse conditions. Glass jars were used, filled with loamy soil. Rainfall was simulated by using an ordinary garden watering pot, the plants being sprinkled every two or three days. As work had to be done in a basement, for lack of other available space, the plants had very little sunshine and did not grow to maturity. Poor horticultural methods probably had much to do with the failure to mature plants. The temperature was ordinary room temperature. In April lettuce and radishes were planted in the open, exposed to natural conditions. On the second day, after planting the seed, the

surface of the ground was sprinkled with a fecal emulsion, mixed with 24-hours' old agar culture of the *Bacillus typhosus*. No nutrient medium whatever was introduced into the infecting mixture. The culture was removed from the agar slant with sterile water and then mixed with the fecal material. Thus the ground was infected subsequent to seeding and prior to the appearance of the plants.

METHOD OF ISOLATION OF THE BACILLUS TYPHOSUS.

Two different media were used for the isolation of the organism, Endo's medium, and a mannite litmus agar. Endo's medium, as ordinarily made, contains 4 per cent agar, alkalinized with 10 cubic centimeters of a 10 per cent solution of sodium carbonate (NA_2 CO_3) to each liter of the neutral agar. If a 10 per cent solution of anhydrous sodium carbonate (NA_2 CO_3) is used in the above quantity, the alkalinity is so great as to be too restraining to the *Bacillus typhosus*. This amount was, therefore, decreased to 6 cubic centimeters to each liter of neutral agar.

The alkaline agar is flasked in amounts of 200 to 400 cubic centimeters. When needed for use it is melted in an Arnold sterilizer, and to it is added 1 per cent lactose, 0.5 per cent saturated alcoholic fuchsin, and 0.25 per cent anhydrous sodium sulphite, according to formula. Plates are poured immediately.

Firth and Horrocks laid great stress on the efficacy of the glucose litmus agar plate method in isolating the *Bacillus typhosus*, there being a difference in the color play in the colonies of these organisms, due to the different sugar-splitting properties of the *Bacillus typhosus*, the *Bacillus coli*, and the soil organisms. I did not have the same success as these authors with this medium, but found a mannite litmus agar highly satisfactory.

Both Endo's medium and mannite medium have points of merit as well as disadvantages. Endo's medium has the advantage of inhibiting a number of organisms other than the *Bacillus typhosus*, leaving the colonies of the *Bacillus typhosus* fairly discrete and isolated. The mannite medium in nowise restrains, and it permits of an overgrowth and confluence of colonies. On the other hand several organisms which present the same characteristics on Endo's medium as colonies of the *Bacillus typhosus* show marked differentiation on mannite medium.

In this work two soil organisms corresponding in a general way culturally with the *Bacillus aurescens*, the *Pseudomonas ovalis*, and the *Bacillus lactis aerogenes* caused a confusion with typhoid colonies when plated on Endo's medium. On mannite litmus agar these colonies were readily differentiated, those of the *Bacillus coli* and *Bacillus lactis aerogenes* being opaque, large, and of a dirty pink color; those of *Bacillus aurescens* reddish opaque; those of *Pseudomonas ovalis* opaque and white, while the colony of *Bacillus typhosus* was a clear amber. The difference is readily accounted for by the different mannite-splitting property of these organisms. Planted in mannite broth fermentation tube for 24 hours the net increase of acidity over the control tube was for the *Bacillus typhosus* equal to an amount of decinormal sodium hydrate solution equivalent to 20 per cent of the volume of the culture, for the *Bacillus aurescens* 10 per cent, *Bacillus coli* 17 per cent. The *Pseudomonas ovalis* was slightly alkaline.

METHOD OF EXAMINATION OF PLANTS.

The parts of the plants selected for examination were the leaves and stems, cut off well above ground, so that the roots with the adherent soil could not enter into the results. The procedure varied between rubbing the leaf and stem directly on surface of plated medium, and washing these parts with a small quantity of broth, the latter being plated without any incubation. Using the "washings" immediately, or after standing for an hour or two, gave better results with fewer saprophytes on the resultant plate than plating out from an incubated specimen.

IDENTIFICATION OF THE BACILLUS TYPHOSUS.

Characteristic colonies on the plates were planted in broth tubes and incubated for 24 hours, at which time a drop of highly agglutinative serum was added. If agglutination occurred, the culture was replated and the resulting growth studied on different media. Confirmation was considered complete if the organism was a motile bacillus, agglutinating in high dilutions, not liquefying gelatine, producing an acidity of milk not exceeding that caused by a known typhoid control, giving negative Indol test, and forming no gas on lactose broth.

ERRORS IN AGGLUTINATION.

In only one instance was there agglutination of an organism that later proved to be other than the *Bacillus typhosus*. A strain of *Bacillus acidi lactici* produced agglutination, but not of the same appearance as that caused by the typhoid strain worked with. Whereas the *Bacillus typhosus* early gave a flocculent precipitate, the upper part of the broth tube becoming clear, this *Bacillus acidi lactici* caused a finely granular appearance in the broth and did not tend to precipitate or become flocculent. Subsequent growth on other media proved its identity.

EXPERIMENT I.

Plants cultivated within house.

Date planted.	Date inoculated.	Parts examined.	Date of last positive examination.	Total longevity.
Jan. 6	Jan. 8	Leaves and upper stems	Feb. 2	25 days.

In this experiment the plants commenced wilting on February 3, and no subsequent examination was made. In all there were during the growth of the plants 10 clear days. The total exposure of the growing plants to sunlight was approximately 30 hours. Examinations were made on alternate days from the time the plants first appeared above the ground. The plants were watered every two days with a garden sprinkling pot.

EXPERIMENT II.

Plants cultivated in open air exposed to rain and sunlight during part of day.

Date planted.	Date inoculated.	Parts examined.	Date of last positive examination.	Total longevity.
Apr. 12	Apr. 15	Leaves and stems	May 16	31 days.

During the period from April 15 to May 16 there were 23 days, with approximately 138 hours of exposure to direct sunlight. On 8 days there was rain, on 4 of which moderate showers fell, and 4 very light precipitation. These vegetables were planted in a location where the sun shone upon them only in the forenoon. Examinations were made every 3 days. Examinations were positive for the *Bacillus typhosus* up to the tenth day. From the tenth day to the thirtieth the organism was not recovered, but on the thirtieth and thirty-first, positive results were obtained. The *Bacillus typhosus* was not recovered after the thirty-first day.

In this examination from 6 to 10 endo plates were made at each examination and from 30 to 40 suspected colonies picked, the large majority of which were negative.

Plants were not sprinkled during this period, although there was practically a drought as only 1.03 inches of rain fell throughout the 31 days. As desiccation is one of the most devitalizing conditions affecting the *Bacillus typhosus*, this test was more severe than would have obtained in most truck gardens where good horticultural methods are in use, as in the latter the moisture due to irrigation or sprinkling would constitute a more favorable environment for the *Bacillus typhosus* and conduce to greater longevity.

The only explanation for negative examinations from the tenth day to the thirtieth day, aside from defective technique, or lack of thoroughness in examination, is that the leaves examined on the thirtieth and thirty-first days were smaller and protected from the sun's rays by a larger plant.

The plant examinations were negative subsequent to May 16.

The *Bacillus typhosus* was recovered from the soil 35 days after inoculation.

EXPERIMENT III.

Plants cultivated in open air exposed to sunlight throughout entire day and to rainfall.

Date planted.	Date inoculated.	Parts examined.	Date of last positive examination.	Total longevity.
Apr. 12..	Apr. 15..	Leaves and stems	Apr. 25..	10 days.

Conditions in this experiment were the same as in the succeeding except as to length of exposure to sunshine. This space was unshaded throughout the entire day. From April 15 to 25 the total exposure to sunshine amounted to 84 hours.

EXPERIMENT IV.

In order to determine with what tenacity the organisms were adherent to the plant and to what degree natural precipitation might be depended upon to free growing vegetables of infected material, a leaf of lettuce, from an infected bed, was subjected to washing. The leaf was placed in a conical glass containing sterile water and thoroughly cleansed by means of a pipette and platinum needle. This was repeated by passing the leaf through two other washings. After the third washing the leaf, in an almost macerated condition, was rubbed on an Endo plate and all three washings were plated out. Plates inoculated from the first washing and from the leaf gave positive findings.

CONCLUSIONS.

In the foregoing experiments it is evident that plants cultivated in contaminated soil will take up on the leaves and stems, as they grow through the soils, organisms existing therein.

The *Bacillus typhosus* was recovered from the tips of leaves that were, to naked-eye appearances, free from soil, although it is presumable that microscopic particles of earth were adherent to the leaves.

Rainfall will not free vegetables from infected material.

Conditions in Experiments II and III correspond to the natural, except as to the infecting material, which was an artificially infected stool, as no typhoid stool was available.

Under conditions most unfavorable to the *Bacillus typhosus*, the infection lasted at least 31 days, a period sufficiently long for some varieties of lettuce and radishes to mature.

SANITARY CONSIDERATION.

Accepting the results of a large number of investigators, it is evident that the *Bacillus typhosus* may be classed among the soil bacteria rather than among the water organisms. The longevity of the *Bacillus typhosus* according to different workers, ranges in unsterilized water from 7 to 30 days, whereas the duration of life is prolonged in soil to 60 and 70 days.

With this end in mind, the fertilization of ground by human excreta assumes a twofold importance. When pollution of garden earth with infected material occurs, not only may the vegetables thereon, such as lettuce, radishes, and celery, directly convey infection, but the soil may serve as a reservoir for the bacteria, drainage from such areas serving to maintain in streams an infection for much longer periods than if the infection of the stream were direct.

Of all the problems confronting the sanitation of this country in recent years few have received more attention than that of stream pollution. But in the consideration of the contamination resulting from the discharge of urban sewage, which is admittedly of paramount importance, that arising from a great and widespread rural population has been given less consideration than it deserves.

The practice of using human excreta as fertilizer is by no means as uncommon as is generally supposed, and without doubt will become more widespread unless this method of soil enrichment be curtailed by properly enforced laws. Although some emphasis has been laid on the fact that tips of vegetables examined were microscopically free from earth, the viability of the *Bacillus typhosus* on plants, and its longevity in soil, can be considered identical so far as the sanitary

significance is concerned, for very seldom if ever is there seen in the market lettuce or celery free from dirt, and even in well managed households and public eating places scrupulous care in preparing articles for the table is exceptional.

BIBLIOGRAPHY.

Stimson, Hygienic Laboratory Bulletin 35, p. 138.
Firth and Horrocks. British Medical Journal, 1902, p. 936.
Galvagne and Calderini. Zeit. f. Hyg. 1908, LXI, 188.
Levy and Kayser Cent. Bakt. 1903, XXXIII, 489.
Mair. Jour. Hygiene, 1908. Vol. 8.
Viability of *B. typhosus* in soil.

TYPHUS FEVER IN THE UNITED STATES.

In last week's issue of the Public Health Reports there appeared under the title "The relation of so-called Brill's disease to typhus fever," a report of work done by Passed Asst. Surgs. Anderson and Goldberger, which shows that the so-called Brill's disease is identical with the typhus fever of Mexico. The typhus fever of Mexico is very probably the same as the typhus fever of Europe and Asia, and presumably the infectión known to exist in New York, and understood to have occurred in other large cities, was imported by European or Asiatic immigrants. This gives the American physician a new disease with the symptoms of which he should familiarize himself, and the possible appearence of which among patients he should keep constantly in mind. To the health authorities of the United States it presents a new infectious disease for consideration and control.

From 1896 to the end of 1910 Dr. Nathan E. Brill noted among the medical patients in one hospital in New York City 255 cases of a disease which in general symptoms resembled typhoid fever to some extent, and which very probably has usually been so diagnosed. Dr. Brill, however, clearly differentiated the two diseases, and in various reports drew attention to the similarity of his cases to typhus fever. That the disease could be typhus fever, however, he could hardly believe, because of its mildness and low fatality. Among the 255 cases which he had observed there was but one death.

In addition to the cases reported by Brill, Dr. Leon Louria reported 18 cases observed during the summer and autum of 1910 in one hospital in Brooklyn.

The fact that cases of typhus fever have been confused with typhoid fever in New York City, and that they are without doubt being so confused in other large cities, is of interest in view of the fact that originally these two diseases were both included under typhus fever and no differentiation was made between them. Gerhard and Pennock, of Philadelphia, are commonly given credit for having in 1837 first definitely established that typhoid fever and typhus were distinctly separate entities.

The clinician has at all times found difficulty in diagnosing mild cases of even the more common diseases. Some of this difficulty is inevitable, but much of it has been due to the fact that the usual descriptions of a disease given in the literature are of its more severe manifestations, which are assumed to be usual and typical and frequently pathognomonic. The natural result of this is that very probably certain diseases are recognized only in their more virulent and at